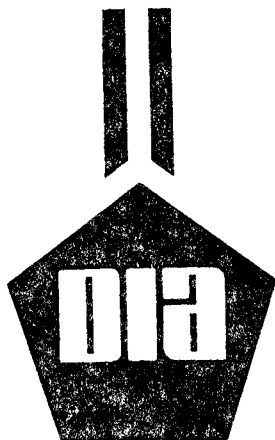


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DIAIAPPR 149-81



DEFENSE
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30 SEP 1981

Intelligence Appraisal
Southeast Asia:
Mycotoxins and the
Soviet Connection(U)

26 SEPTEMBER 1981

DIA Review

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Intelligence Appraisal

SOUTHEAST ASIA: MYCOTOXINS AND THE SOVIET CONNECTION(U)

PREPARED BY

Dr S Watson

USAMIA

This is a Department of Defense Publication
Produced by the Defense Intelligence Agency

Classified by Multiple Sources
Review on 16 Sep 01

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NATURAL OCCURRENCE OF TRICHOHECENES

<u>TOXIN</u>	<u>SAMPLE</u>	<u>DISTRICT</u>	<u>CONTENTS</u>
T2 TOXIN	CORN	WISCONSIN	2 PPM
DEOXYNIVALENOL	CORN	OHIO	3 PPM
DEOXYNIVALENOL	CORN	ILLINOIS	8 PPM
DEOXYNIVALENOL	CORN	INDIANA	8 PPM
DEOXYNIVALENOL	CORN	KAGAWA, JAPAN	4 PPM

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SECRET**SOUTHEAST ASIA: MYCOTOXINS AND THE SOVIET CONNECTION (U)****Summary**

(S) As a result of a current interagency collection and analysis program, a sample of vegetation, obtained soon after an alleged chemical warfare (CW) attack in Kampuchea near the Thailand border, was recently tested for the presence of trichothecene toxins. Unusually high levels of three trichothecene toxins were identified by a complex testing protocol developed by an internationally recognized mycotoxin expert. Extensive searches of published scientific and technical literature indicate that the fungi capable of producing these toxins are not known to produce these toxins under the climatic conditions in Southeast Asia. Thus, the high levels of these toxins strongly indicate a nonnatural occurrence. Additionally, a number of the symptoms reported by numerous refugee are also characteristically caused by the trichothecene toxins. Soviet experience, technical expertise, and production capabilities have been reviewed and are strongly suggestive of a Soviet connection with the alleged CW attacks that have been reported since the mid-1970s.

Discussion

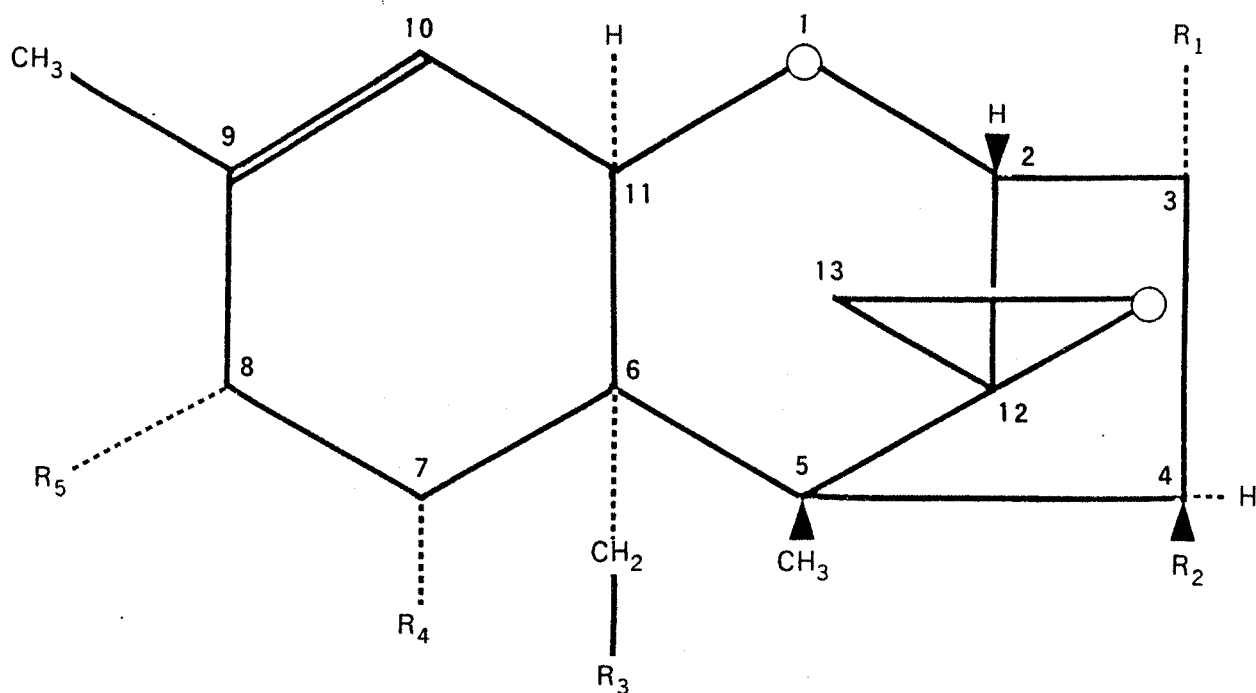
(S) Analyses of a vegetation sample allegedly exposed to a CW attack near the Thai-Kampuchean border, have revealed the presence of trichothecene toxins in abnormally high concentrations. These analyses were performed on a "blind" basis by the leading US expert in detection of such toxins. The alleged CW sample (code sample A), a positive control containing a known quantity of trichothecene (code sample B), and a negative control sample (code sample C) were submitted for analysis. The expert was given no information concerning the history or content of the samples, but was requested to analyze three unknowns (A, B, and C) for the presence of the toxins by the best state-of-the-art methods available.

(S) The methods used were the most sensitive yet developed for detection of these compounds and, even more importantly, were the most specific. The incidence of false positives with these techniques is essentially nonexistent. The toxins can be positively identified by their mass spectra and can also be quantified with a high degree of accuracy. Sample A (the vegetation sample allegedly exposed to a CW agent) was found to contain 109 parts per million (ppm) of nivalenol, 59.1 ppm of deoxynivalenol, and 3.15 ppm of T2 toxin -- all potent toxins of the trichothecene group. No trichothecenes were detected in the negative control sample C, and 35 ppm of T2 toxin were detected in sample B (the sample to which T2 toxin was added). Trichothecenes are mycotoxins or toxins produced by molds. Although mycotoxins have been implicated in numerous natural outbreaks of disease throughout history, the presence of these particular mycotoxins in South Asia is highly unusual. Climatic conditions conducive to the growth of the mold-producing species of *Fusarium* and production of these toxins include very low temperatures. In fact, maximum production occurs at temperatures just above freezing. These trichothecenes are not known to occur naturally in Southeast Asia. The levels of nivalenol and deoxynivalenol detected in sample A were also highly unusual; many times those typically occurring in natural intoxications.

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THE TRICHOTHECENES



T ₂ Toxin	Nivalenol	Deoxynivalenol
R ₁ =OH	R ₁ =CH	R ₁ =OH
R ₂ =OAc	R ₂ =CH	R ₂ =H
R ₃ =OAc	R ₃ =OH	R ₃ =OH
R ₄ =H	R ₄ =OH	R ₄ =OH
R ₅ =OCOCH ₂ CH(CH ₃) ₂	R ₅ =O	R ₅ =O

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(S) According to the mycotoxin analysis expert, it is highly unlikely that such levels could have occurred in a natural intoxication. The three toxins detected are similar in structure and cause similar symptoms that include a rapid onset of vomiting, multiple hemorrhage of mucous membranes, diarrhea, and severe itching or tingling of the skin with the formation of small blisters. Slight differences in their symptomology, however, do exist. For example, nivalenol is more toxic overall than T2 and causes a greater hemorrhagic effect, but is not as strong a skin irritant. Deoxynivalenol is less toxic than the other two, but produces severe vomiting at low dosage levels. In fact, its common name is vomitoxin. Both nivalenol and deoxynivalenol are extremely difficult to detect by routine screening methods. The ketone group of carbon 8 masks the characteristic fluorescence that is typically used to detect the trichothecenes in thin-layer chromatography techniques. The gas chromatography-selected, ion-monitoring, mass-spectral method used in this analysis is required to detect the compounds. So, if one wished to design a mixture of toxins that would be highly effective and difficult to detect, this one (trichothecenes) would be ideal. It is also possible, however, that this particular mixture could have occurred fortuitously.

(U) As in the Trichothecene chart, there is a species of Fusarium, F. roseum that can produce all three of these toxins. If a crude extract from the culture of this species were used as an agent, such a mix could occur. In fact, it would be more economical to use such a culture extract without separating out the component toxins than to obtain each in pure form. It is noted that these three toxins have never been proved to have been produced simultaneously and have never been isolated previously in the same sample.

(U) Trichothecene mycotoxins, perhaps more than any other mycotoxins, have been associated with disease outbreaks in humans. As in the historical chart on trichothecene mycotoxicosis, most of these human intoxications occurred in the Soviet Union. As a result, trichothecenes have figured prominently in Soviet scientific literature since the 1940s. The earliest reported outbreak of the disease caused by these toxins occurred in 1891 in the Ussuri District of eastern Siberia: stored grain was infected with toxin-producing Fusarium. Humans who consumed the contaminated grain experienced headache, chill, severe nausea and vomiting, vertigo, hemorrhage, diarrhea, and visual disturbances. Dogs, horses, pigs, and domestic fowl were also affected.

(U) The most severe outbreak of mycotoxin disease reported to have caused multiple fatalities also occurred in the Soviet Union. In 1944, 30 percent of the population of Orenburg, in the southern Urals, was affected by alimentary toxic aleukia (ATA), a disease later proved to be caused by ingestion of trichothecene toxins. Over 10 percent of the entire population of the Orenburg District died of the disease. Numerous other outbreaks of ATA occurred in the Soviet Union, primarily between 1942 and 1947. The source of contamination was traced to overwintered millet, wheat, and barley infected with Fusarium. Symptoms of the disease included vomiting, skin inflammation, multiple hemorrhaging (especially of the lung and gastrointestinal tissue), diarrhea, leukopenia, and exhaustion of bone marrow.

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HISTORICAL TRICHOTHECENE MYCOTOXICOSIS

<u>TOXICOSIS</u>	<u>DISTRICTS AND AFFECTED SPECIES</u>	<u>SYMPTOMS</u>
"Taumelgetreide" Toxicosis	USSR man, farm animals	Headache, nausea, vomiting, vertigo, chills, visual disturbances.
Alimentary Toxic Aleukia	USSR man, horse, pig	Vomiting, diarrhea, multiple hemorrhage, skin inflammation, leukopenia, angina.
Stachibotryotoxiosis	USSR, Europe horse	Shock, somatitis, hemorrhage, dermal necrosis, nervous disorders.
Bean-hull Toxicosis	Japan horse	Convulsion, cyclic movement.
Dendrodochio toxicosis	USSR, Europe horse	Skin inflammation, Hemorrhage.
Moldy Corn Toxicosis	US pig, cow	Emesis, hemorrhage.
Red Mold Toxicosis	Japan, USSR man, horse, pig, cow	Vomiting, diarrhea, congestion and hemorrhage of lung and intestine.

TOXICOLOGICAL CHARACTERISTICS OF
TRICHOTHECENE MYCOTOXINS

1. Vomition, emesis, tachycardia, diarrhea.
2. Hemorrhage of mucosal epithelia of stomach and intestine.
3. Hemorrhage, edema, and necrosis of skin.
4. Destruction of hematopoietic tissues.
5. Decrease of circulating white blood cells and platelets.
6. Meningeal hemorrhage in brain.
7. Nervous disorders.

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(U) Other disease outbreaks in which similar symptoms were exhibited occurred in 1958 and 1959 in horses and cattle in the Soviet Union and Eastern Europe. Thousands of animals died in this outbreak. Other intoxications were reported subsequently in Japan, Europe, the Soviet Union, and the US. These affected various domestic animals and, in the case of red mold toxicosis, man. All of these diseases have now been proved to be caused by ingestion of trichothecenes, rather than an infectious agent. The levels of toxin that were detected in toxic grains implicated in "moldy corn toxicosis" and in "red mold toxicosis" were typically between 2 and 8 ppm.

(C) The symptoms commonly associated with trichothecene poisoning are listed in the Toxicological Characteristics of Trichothecene Mycotoxins chart. Striking among these symptoms is the rapid onset of vomiting and hemorrhage of the mucous membranes, as well as severe itching or tingling of the skin with formation of small blisters. These are similar to the symptoms reported by the H'Mong tribesmen after attacks with the "yellow rain" in Laos. From an interview with a Lao refugee in Thailand, who claimed to be a victim of a chemical attack that occurred in May of 1977, the following was taken. After exposure to the agent, the victim immediately felt dizzy and nauseous; experienced frontal headache, vertigo, painful burning of eyes and throat; had difficulty breathing and was extremely nauseated -- vomiting blood on several occasions; and suffered from bloody diarrhea and his skin itched all over, developing small blisters and numerous hemorrhages under the skin. The symptoms described are very similar to those described for ATA and other classical trichothecene intoxications. Also of interest in this context is a recent intelligence report providing detailed information concerning a Cuban CW training exercise. Two water contamination agents were demonstrated. A soldier who removed his mask during the demonstration was contaminated by the fine powder of one of the agents. He immediately experienced severe eye irritation and uncontrollable itching of exposed skin and vomiting with apparent hemorrhage of mucous membranes followed. Again, these symptoms are quite similar to those associated with trichothecene poisoning.

(U) Even though the symptoms exhibited by alleged victims of these intoxications in Southeast Asia are similar to those produced by trichothecenes and the Soviets are clearly aware of the existence of these compounds, any association between the Soviets and the use of such agents in Southeast Asia is circumstantial. Further analysis leads to several questions, which should be addressed:

1. Are the chemical properties of these compounds suited to their use as agents?
2. Do the Soviets have the expertise to produce the large quantities that would be needed for such operations?
3. What are the indications that Soviet research projects with these compounds are associated with the military?
4. What are the indications of Soviet involvement in the suspected agent attacks?

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(U) When considering suitability of the toxins as agents, factors such as stability, solubility, and toxicity must be considered. The general structure for the trichothecene group is shown in the Trichothecene chart. There are 37 presently known, naturally occurring, 12-13 epoxy-trichothecenes. The R groups may be hydroxyls, acylated hydroxyl groups, or esters. The R group for the toxins detected in sample A are shown below the general structure. All of the compounds have in common an olefinic double bond at carbon atoms 9 and 10 and an epoxy group at carbon atoms 12 and 13. These compounds are stable, especially in the solid form. They may be stored for years at room temperature with no loss of activity. They are heat stable with no loss of activity noted after heating for 1 hour at 100 degrees centigrade. The solubility depends on the R groups; highly hydroxylated derivatives are more water soluble. The compounds are also quite stable in solution. Detoxification requires treatment with strong mineral acid, which will open the 12-13 epoxide bond and abolish all biological activity. Most of the toxins are well absorbed through mucous membranes and some through skin, however, this property is also a function of the R groups. The LD 50s -- lethal dose required to cause death in 50 percent of the exposed population -- of the toxins in laboratory animals range from 0.1 milligram per kilogram (mg/kg) to 150 mg/kg. The LD 50 for T2 toxin is 5.2 mg/kg in rats. However, the ED 50 -- effective dose is the amount of chemical agent required to produce desired physiological effect in 50 percent of the exposed population -- for the vomiting reaction is 0.1 mg/kg and for skin irritation it is in the tenths of a microgram range. The hemorrhagic effect produced by the compounds is due to a primary defect in Factor VII (clotting factor) activity and secondary effects on prothrombin. These compounds are immunosuppressive. They produce a severe leukopenia, which may persist as long as 20 days after exposure. In general, the toxicological and chemical properties of these toxins would make their use as agents theoretically possible, but the question remains whether they could be produced in the required quantities.

(U) Some of these compounds have been synthesized chemically; however, biosynthesis employing Fusarium species is the most effective way to produce large quantities. In a preliminary search of recent Soviet open source literature, 50 articles dealing with the trichothecenes were reviewed. Of these, 22 dealt with defining optimum conditions for biosynthesis of the compounds. N.A. Kostyunina has reported production of T2 toxin at levels of 4 grams per kilograms of substrate (normally wheat, grain, or rice). Numerous industrial microbiology plants have been identified in the Soviet Union. Some of these are involved in production of single-cell protein for fodder additives, others produce antibiotics, and the function of still others is unknown. The only difference between an antibiotic and mycotoxin is their specificity of target. Both are produced by molds but the mycotoxins are toxic to man as well as to micro-organisms. Mycotoxins can be produced in good yield employing the same techniques that are used to produce some antibiotics. Thus, it is concluded that the Soviets could produce trichothecenes in large amounts. They produce an antibiotic that is a trichothecene derivative, which would provide an ideal cover for agent production facilities.

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USSR INSTITUTES INVOLVED IN RESEARCH WITH FUSARIUM TOXINS

Institute of Microbiology and Virology, Kiev.

- * All Union Scientific Research Institute of Experimental Veterinary Science, Moscow.

All Union Scientific Research Institute of Veterinary Sanitation, Moscow.

All Union Research Institute of Grain and Grain Products.

- * Institute of Epidemiology and Microbiology Imeni N. F. Gamaleya, Moscow.

Institute of Nutrition, Academy of Medical Sciences.

- * All Union Research Institute of Microbial Means for Plant Protection and Bacterial Preparations.

- * Institute of Pharmacology, Zubovskaya Ploshchad, Moscow.

Institute of Biological Testing of Chemical Products, Kupavna, (a suburb of Moscow).

Institute of Chemistry of Natural Compounds, Moscow.

- * Linked with highly classified CBW research Projects.

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(C) In considering the question of a military link of researchers of institutes conducting trichothecene research, a major gap in information is encountered because there were no intelligence collection requirements in the field until recently. It is known that extensive research in this area is being conducted in the Soviet Union. This fact is evident from the conducted literature searches. In 1977, at the Institute of Microbiology and Virology in Kiev, a week-long "Fusarium school" was held to examine the effects of the toxins and define conditions of their production. Later that year at Orenburg, a symposium was held entitled "Mycotoxins, Producers, Chemistry, Biosynthesis, and Effects on the Organism." Some 550 scientists from many different Soviet institutes participated. At that time, it was recommended that a Central Coordinating Interdepartmental Commission be created by the Presidium of the USSR. A chart identifies some of the institutes involved in research with Fusarium toxins. Those institutes that have been previously linked with highly classified chemical and biological warfare (CBW) research projects are starred.

(S) Furthermore, specific information exists concerning military-funded, highly classified projects involving another mycotoxin, aflatoxin B. This toxin is listed in a 1977 East German military manual, entitled Chemical Warfare Agents and Protection Against CW Agents, as a potential sabotage agent. Especially disturbing regarding this fragmentary information is the statement made in 1978 by the head of the Polish chemical troops that toxins were the third generation of chemical weapons. He referred to mustards as the first generation, nerve gases as the second, and indicated that toxins may be the weapon of the future.

(S) There is a striking similarity in the symptoms experienced by surviving victims in Laos and Kampuchea following attacks with "yellow power" and those associated with mycotoxicosis. Very similar descriptions of attacks and symptoms have also been reported in Afghanistan, where the Soviets are more directly involved.

(S) To summarize concerning the trichothecenes, the Soviets are quite familiar with the toxins and have considerable experience in their chemistry. The symptoms of the "alleged gassing" victims in Laos and Kampuchea, as well as in Afghanistan, correspond with those observed in trichothecene poisoning. The chemical and toxicological properties of the trichothecenes would make their use as agents feasible. The Soviets have the capability to produce large amounts of the toxins and to conceal that production under the cover of antibiotic production. Soviet institutes previously linked to classified CBW-research projects are also involved in such projects concerning the trichothecenes. And finally, a vegetation sample alleged to have been exposed to a CW agent in Kampuchea contained abnormally high levels of trichothecene toxins. Although the evidence is somewhat fragmentary, when taken as a whole, a very strong circumstantial case is made for the allegation that trichothecene toxins have been and are being employed as agents in Southeast Asia. There is a probable connection with the Soviets in Laos and Kampuchea, where there is evidence they are functioning in a technical advisory and supply role. Although results of one sample analysis do not constitute conclusive proof, detection of the same trichothecenes in other samples, which can be attributed with a degree of certainty as coming from an area that was subjected to CBW attack, will provide confirmatory evidence. Additional analyses of such samples are currently underway.

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Outlook

(S) The identification of trichothecene toxins introduces a number of medical defense and protection problems because there is no known effective prophylaxis. Only the symptoms can be alleviated. These are extremely difficult toxins to neutralize using available standard methods for conventional CW agents. It is also important to note that their identification does not mean that other types of CW agents have not been or are not being used.

(S) Acquisition and analysis of samples will continue to be complicated and troublesome because of the remoteness of the areas of the alleged CW attacks. However, efforts will continue to obtain multiple samples that are randomly taken and are representative of the areas of the alleged CW attacks. (Classified by multiple sources; review 16 Sep 01)

Contributions by:

Mr D. Gasbarri, DT-1A

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